Pharmacokinetics and tolerability of inhaled levodopa from a new dry-powder inhaler in patients with Parkinson's disease

Luinstra, Marianne; Rutgers, Wijnand; van Laar, Teus; Grasmeijer, Floris; Begeman, Anja; Isufi, Valmira; Steenhuis, Luc; Hagedoorn, Paul; de Boer, Anne; Frijlink, Henderik W.

Published in:
Therapeutic advances in chronic disease

DOI:
10.1177/2040622319857617

Ther Adv Chronic Dis
2019, Vol. 10: 1–10
DOI: 10.1177/2040622319857617

Pharmacokinetics and tolerability of inhaled levodopa from a new dry-powder inhaler in patients with Parkinson's disease

Marianne Luinstra, Wijnand Rutgers, Teus van Laar, Floris Grasmeijer, Anja Begeman, Valmira Isufi, Luc Steenhuis, Paul Hagedoorn, Anne de Boer and Henderik W. Frijlink

Abstract

Background: Inhaled levodopa may quickly resolve off periods in Parkinson's disease. Our aim was to determine the pharmacokinetics and tolerability of a new levodopa dry-powder inhaler.

Methods: A single-centre, single-ascending, single-dose–response study was performed. Over three visits, eight Parkinson's disease patients (not in the 'off state') received by inhalation 30 mg or 60 mg levodopa, or their regular oral levodopa. Maximum levodopa plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}) and area under the concentration time curve 0–180 min were determined. Spirometry was performed three times at each visit.

Results: After inhalation, levodopa T_{max} occurred within 15 min in all participants, whereas after oral administration, T_{max} ranged from 20 min to 90 min. The bioavailability of inhaled levodopa without carboxylase inhibitor was 53% relative to oral levodopa with carboxylase inhibitor. No change in lung-function parameters was observed and none of the patients experienced cough or dyspnoea. No correlation was observed between inhalation parameters and levodopa pharmacokinetic parameters.

Conclusion: Inhaled levodopa is well tolerated, absorbed faster than oral levodopa, and can be robustly administered over a range of inhalation flow profiles. It therefore appears suitable for the treatment of off periods in Parkinson's disease.

Keywords: inhaled levodopa, levodopa dry-powder inhalation, off periods, Parkinson's disease

Received: 28 March 2019; revised manuscript accepted: 23 May 2019.

Correspondence to:
Floris Grasmeijer
Department of Pharmaceutical Technology and Biopharmacy, University of Groningen, Antonius Deusinglaan 1, Groningen 9713 AV, The Netherlands
f.grasmeijer@rug.nl

Marianne Luinstra
Department of Clinical Pharmacy, Martini Hospital Groningen, Groningen, The Netherlands

Department of Pharmaceutical Technology and Biopharmacy, University of Groningen, Groningen, The Netherlands

Wijnand Rutgers
Department of Neurology and Clinical Neurophysiology, Martini Hospital Groningen, Groningen, The Netherlands

Teus van Laar
Department of Neurology, University Medical Centre Groningen, Groningen, The Netherlands

Anja Begeman
Valmira Isufi
Paul Hagedoorn
Anne de Boer
Henderik W. Frijlink
Department of Pharmaceutical Technology and Biopharmacy, University of Groningen, Groningen, The Netherlands

Luc Steenhuis
Department of Pulmonary Diseases, Martini Hospital Groningen, Groningen, The Netherlands

Introduction

Parkinson's disease is characterized by the degeneration of dopaminergic neurons in the substantia nigra in the brain.[1] The resulting lack of dopamine in the brain causes disruption of brain circuits, thereby provoking the core motor features of bradykinesia plus rest tremor or rigidity.[2] Levodopa, nonergot dopamine agonists and monoamine oxidase (MAO) inhibitors are effective in relieving the motor symptoms and signs of the disease.[3] Levodopa is administered *via* the oral or duodenal route, and dopamine agonists are administered *via* the oral, transdermal or subcutaneous route. Several years after being diagnosed with Parkinson's disease, many patients develop motor fluctuations as a result of a narrowing therapeutic window of levodopa[4] in combination with a delayed onset of effect after orally administered levodopa due to irregular gastrointestinal absorption.[5] This may lead to 'off periods', in which Parkinson's symptoms are poorly controlled[6] and patients suffer from a variety of complaints such as bradykinesia, decreased mobility, tremor and autonomic or sensory symptoms.[7] For patients with severe motor fluctuations on oral levodopa therapy, the only registered drug for termination of the off periods is subcutaneous apomorphine. After

injection, an onset of effect of apomorphine is generally not seen within a 20-min lag time.[8] Since being in an off period causes a great burden to the patient, a rapid onset of the effect is desired. Unfortunately, apomorphine is a strong emetic, causing nausea and vomiting on a regular basis. Patients using apomorphine therefore frequently require antiemetic drugs.[9] Another disadvantage of apomorphine is its administration *via* (self) injection. In spite of improved needle technology, injection is considered burdensome by many patients.

An alternative strategy in daily practice for ending an off episode is the oral administration of levodopa/benserazide dispersible tablets. Faster effect than standard levodopa formulations is expected. For dispersible levodopa to be effective, the levodopa needs to be absorbed *via* the small intestine. It is known that most off symptoms improve within about 30–60 min after administration of dispersible levodopa, but in some patients, improvement of symptoms is delayed or does not occur at all.[10] After oral administration, the absolute bioavailability of immediate-release levodopa is 40–60%, combined with a decarboxylase inhibitor, it raises to approximately 85%.[11]

The C_{max} is reached after 1 h on average. However, it is known that food, especially proteins, decreases the absorption rate of levodopa. Food also increases the time to the C_{max}. Levodopa is metabolized mainly *via* decarboxylation by the aromatic amino acid decarboxylase to dopamine, adrenaline, and noradrenaline and *via* O-methylation by catechol-O-methyltransferase (COMT) and MAO.[12,13,14] Levodopa used in combination with a decarboxylase inhibitor has a relatively short plasma half-life time of approximately 90 min.[15]

Delivery of systemically acting drugs by inhalation can offer various advantages compared with their oral administration.[16,17] After correct pulmonary administration, a major portion of the drug is immediately deposited on the absorbing membrane, which results in rapid absorption compared with oral administration. The drug is not subjected to the drug-metabolizing enzymes and efflux transporter activity of the gut and first-pass metabolism of the liver that occurs after oral administration.[16] Moreover, administration of levodopa by inhalation bypasses the irregular gastrointestinal absorption of levodopa in off periods, which is caused by irregular gastrointestinal motility. In contrast, after inhalation, small molecules can be absorbed within seconds to minutes,[16] which has been confirmed for levodopa by Lipp *et al*.[18] They showed that after inhalation of their levodopa formulation CVT301, the drug was rapidly absorbed into the bloodstream. Already 5 min after receiving 50 mg of CVT-301, 67% of the participants showed a levodopa plasma concentration over 400 ng/ml. This rapid absorption clearly is an advantage when a quick response of a drug is desired, as in off periods in Parkinson's disease. Pulmonary administration of levodopa is therefore considered a promising alternative to injected apomorphine or to dispersible levodopa tablets. This promise is further strengthened by the fact that Parkinson's disease patients are generally capable to perform a correct inhalation manoeuvre during an off period.[19]

So far, the only described dry-powder inhalation system for levodopa is the CVT301.[18] The CVT301 system is based on a spray-dried powder containing only 50% levodopa, with 25% dipalmitoyl phosphatidylcholine, 15% sodium citrate, and 10% calcium chloride as excipients. The high load of excipients increases the amount of powder to be inhaled and may lead to side effects, such as cough. Motivated by the positive results from our study on inhalation manoeuvres, we developed a new dry-powder inhalation system for levodopa that contains 98% pure crystalline drug and only a minor amount (2%) of an endogenous excipient.[20] Furthermore, the fact that this formulation contains only crystalline levodopa is expected to improve the stability of the final product. Being a preloaded, disposable inhaler, the Cyclops® **(PureIMS, Roden, the Netherlands)** does not require the loading of capsules, contrary to CVT301, which makes it easier to use. Furthermore, the high resistance to airflow of the Cyclops may minimize the chance of cough reactions during inhalation.

In this article, we present the pharmacokinetic data of an unblinded single-centre, single-ascending, single-dose–response study of a pulmonary administered 30 mg and 60 mg levodopa with 2% L-leucine dry-powder dose in Parkinson's disease patients. Besides the pharmacokinetic evaluation of inhaled levodopa, the tolerability of the airways

for inhaled dry-powder levodopa was assessed using spirometry data. Furthermore, by recording the inhalation flow profiles during dose administration we examine the relationship between inhalation and pharmacokinetic parameters.

Materials and methods

Materials

Levodopa, European Pharmacopoeia quality, was supplied by Duchefa Farma (Haarlem, The Netherlands). L-leucine was purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). The levodopa was blended with 2% w/w L-leucine and micronized. Subsequently, the powder was weighed into blisters. Each blister contained 30.3 mg powder, corresponding to 30 mg levodopa. The inhaler used in this trial was the Cyclops® dry-powder inhaler (DPI).[21]

Informed consent and ethics

All procedures performed in this study were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments. The study protocol was approved by the official Dutch ethics committee 'Regionale toetsingscommissie patiëntgebonden onderzoek' (RTPO) in Leeuwarden, The Netherlands (approval number RTPO949). All participants provided written informed consent for their participation in this study. The study was registered in the Dutch trial register www.trialregister.nl (5435). The study was carried out in concordance with the International Council for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines for Good Clinical Practice.

Study population

Eight participants with Parkinson's disease were recruited from the outpatient clinic of the Department of Neurology and Clinical Neurophysiology of the Martini Hospital Groningen, the Netherlands. The study was performed between October 2016 and March 2018.

The sample size calculation is based on the expected levodopa plasma concentration after 10 min, since a rapid onset of effect is desired. Inhalation of 30 mg of CVT301 results in a plasma levodopa concentration of 425 ng/ml with a standard deviation of 95 ng/ml after 10 min. After administration of 100 mg of oral levodopa in the fasted state, this value is around 150 ng/ml. With a power of 0.8 and a type I error rate α of 0.05, the required sample size would be two study participants. Because of expected differences between CVT301 and the inhaler used in this study, a larger sample size of eight participants was assumed to be sufficient.

Patients were eligible if they were at least 18 years of age; diagnosed with Parkinson's disease; currently on a stable levodopa regime with a maximum of four administrations per day and able to perform spirometry. Participants were excluded if they met one or more of the exclusion criteria. Exclusion criteria were: cognitive dysfunction that precludes good understanding of the instructions; being pregnant or breastfeeding; being known to suffer from active pulmonary disease; symptomatic orthostatic hypotension or using a COMT or MAO-B inhibitor.

Dosing

Over three visits, at least 7 days apart, the participants received a 30 mg inhalation powder levodopa dose (visit one), a 60 mg (2 $\times$ 30 mg) inhalation powder levodopa dose (visit two) and their regular oral levodopa dose (visit three). The inhaled levodopa doses were chosen such that they would remain well below the acceptable single oral dose of 250 mg, assuming a fourfold dose advantage by inhalation. The oral levodopa/decarboxylase dose varied between 100/25 mg and 250/62.5 mg.

All visits and study-drug administration took place in the morning. All patients took their regular breakfast at home, of which the composition details were not collected. The participants were not allowed to take any food or drinks (except water) in the period 60 min before until 60 min after administration of the levodopa.

All levodopa doses administered in this study were at least 7.5 h after the last levodopa administration at home, which is five times the half-life time of levodopa plus decarboxylase inhibitor. The time of the last levodopa administration at home, the time of inhalation of the levodopa

powder and the time of oral administration of the levodopa in this study were recorded in case report forms.

Levodopa inhalation

The levodopa inhalation powder was administered by inhalation through the mouth. Prior to levodopa inhalation, a lung technician trained participants in the correct handling of the inhaler device, including the different steps of a correct inhalation manoeuvre. For this training, an empty, instrumented inhaler was used. For measuring the flow curves through the inhaler, a differential pressure gauge (PD1 with MC2A measuring converter) was used (Hottinger, Baldwin Messtechnik, Darmstadt, Germany). The pressure drop across the inhaler was computed into a flow rate, using a laptop loaded with LabViews software (National Instruments BV, The Netherlands).

The inhaler used for the levodopa administration was instrumented in the same manner. The generated flow curves were shown to the patient on the computer screen during training, as well as during inhalation of the levodopa, to enable the patient to adjust the desired inhalation effort. On the screen, the minimal required and maximal desired flow rate were indicated. The obtained flow curves during inhalation of the levodopa were also used to compute the inspiratory peak flow rates and inhaled volumes. For the 60 mg dose, the inhalation parameters of the first and second inhalation were averaged to enable further calculations. Because these parameters affect the dose emission from the inhaler, the aerosol generation process, as well as the lung deposition, they are a potential source of variation in the inhaled dose and its lung deposition. Hence, their evaluation potentially allows for the explanation of unexpected pharmacokinetic results. After inhalation of the levodopa, the inhaler residue was determined by ultraviolet spectrophotometric analyses (UV-1800 spectrophotometer Shimadzu Benelux, The Netherlands). The delivered dose was subsequently calculated from blister dose minus inhaler residue. The fine particle dose ($<5\,\mu m$), being 45% of the delivered dose at 4 kPa, was determined with a Sympatec HELOS BF laser diffractometer (Sympatec, GmbH, Clausthal-Zellerfeld. Germany).

Blood sampling

Blood samples were collected before administration of the levodopa ($T = 0$) and at $T = 5$, 10, 15, 20, 30, 45, 60, 90 and 180 min after administration of the levodopa. The exact time of blood sampling was noted in the case report forms. Sampling was performed using an intravenous (IV) line filled with saline to avoid blood clotting of the system. To avoid dilution of the blood samples with saline, every first tube was rejected and every second tube was used for analysis. In case of problems with the IV line, a blood sample was drawn by venepuncture. The samples were collected in an ethylenediaminetetra-acetic acid tube. A research nurse took the blood samples.

Analytical methods

After collection, the samples were centrifuged for 12 min at 2500 rpm. The plasma was then transferred to a Sarstedt cup with screw cap. For each ml of plasma, 10 mg reduced glutathione was added to prevent the degradation of levodopa. The samples were stored at −80°C until analysis. Levodopa concentrations were determined using liquid chromatography–tandem mass spectrometry (XLC-MS/MS). The limit of quantification was 1.0 nmol/l.

Spirometry

Spirometry was used to assess the tolerability of the airways for the inhaled dry-powder levodopa formulation. Spirometry was performed before administration of levodopa and ±35 and 100 min after administration of levodopa, respectively. Forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC) and maximum expiratory flow after 50% of the expired FVC (MEF50) were measured using a pneumotachograph.

Spirometry was performed under the guidelines specified by the American Thoracic Society/European Respiratory Society.[22,23] An FEV1 drop $> 10\%$ compared with baseline FEV1 was considered clinically relevant. Additionally, active questioning for cough and dyspnoea was performed during each spirometry session using the Borg Rating of Perceived Exertion.[24] Spirometry was performed by trained lung technicians.

Pharmacokinetic evaluation

The linear trapezoidal method was used to calculate the area under the concentration time curve (AUC) from $T = 0$ to $T = 180$ min (AUC 0–180). GraphPad Prism 7.0 (La Jolla, California USA) was used to calculate the AUCs. The maximum levodopa plasma concentration (C_{max}) and the time to maximum plasma concentration (T_{max}) were gathered from the obtained concentration time curves. The terminal elimination half-life ($T½$) was computed from the following equation:

$$T1/2 = T * \frac{\ln 2}{\ln \frac{C\max}{Ct}} \qquad \text{Equation 1}$$

T is the T_{max} minus the last timepoint of blood sampling; Ct is the last measured concentration in the concentration time curve and C_{max} the maximum plasma concentration from the concentration time curve. The relative bioavailability from inhalation compared with that from oral administration was calculated as:

$$Frel = \frac{FA}{FB} = \frac{\frac{AucA}{AucB}}{\frac{DoseB}{DoseA}} \qquad \text{Equation 2}$$

where A refers to inhaled levodopa and B to oral levodopa, respectively.

Study objectives, design and study site

The primary objective of this study was the pharmacokinetic evaluation of inhaled levodopa dry powder in comparison with oral levodopa.

The secondary objective was to assess the tolerability of the airways for inhaled dry-powder levodopa using spirometry data as a measure. The study was performed in the Martini Hospital Groningen, The Netherlands.

Results

Patients and data

A total of eight patients were included in the study. Patient characteristics are presented in Table 1. From these patients, 232 blood samples were collected and analysed for levodopa plasma concentrations. Eight samples were missed due to issues with the IV line. The range of time spans between the moments of last levodopa administration at home and administration of the study drug was 14–18 h (minimum/maximum). The delivered doses from the inhaler (all doses, all patients) were quite consistent and were on average 85.3% of the nominal dose (relative standard deviation = 5.6%; Table 2).

Table 1. Patient characteristics.

Characteristics		(*n* = 8)
Age (years)	50–59, *n* (%)	2 (25.0)
	60–69, *n* (%)	2 (25.0)
	70–79, *n* (%)	4 (50.0)
	Mean (SD)	67.9 (8.7)
Sex	Male, *n* (%)	6 (75.0)
	Female, *n* (%)	2 (25.0)
BMI (kg/m^2)	Mean (SD)	27 (2.8)
Hoehn & Yahr score	1.5, *n* (%)	1 (12.5)
	2, *n* (%)	7 (87.5)
Oral levodopa dose (mg)	100, *n* (%)	4 (50.0)
	150, *n* (%)	3 (37.5)
	250, *n* (%)	1 (12.5)

BMI, body mass index; SD, standard deviation.

Levodopa pharmacokinetic data

The Figures 1(a) and 1(b) show the levodopa plasma concentrations after pulmonary administration of 30 (a) and 60 mg (b) of levodopa, of which the C_{max} values doubled approximately from 229.2 ± 62.1 ng/ml to 476.2 ± 132.7 ng/ml, respectively.

Plasma concentrations after oral administration of levodopa are shown in Figure 1(c). For easy comparison of the plasma levodopa concentrations after oral administration, all administered oral doses (varying from 100 to 250 mg) were recalculated into plasma concentrations per 100 mg oral levodopa.

Table 2. Summary of plasma pharmacokinetic parameters of inhaled levodopa.

Participant	Delivered dose (mg)		C_{max} (ng/ml)		T_{max} (min)		T ½ el (min)		AUC 0–180 (min*ng/ml)	
	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2
1	23.6	54.2	159.5	567.2	5	10	65.5	48.9	11,712	32,755
2	26.4	52.4	337.0	584.7	10	10	49.2	42.7	15,825	28,953
3	25.3	52.6	228.6	628.2	10	15	58.8	55.2	13,676	38,130
4	26.0	52.2	307.8	446.5	15	15	34.0	76.9	21,541	41,475
5	22.5	47.2	211.0	574.6	15	10	68.1	36.6	15,214	26,340
6	25.8	54.1	188.1	418.9	10	10	57.5	46.4	12,219	24,365
7	25.8	47.8	182.4	308.2	10	5	57.2	62.4	11,904	21,125
8	26.1	52.6	218.8	281.3	5	5	76.3	92.9	15,085	24,072
Mean	25.2	51.6	229.2	476.2	10	10	58.3	57.8	14,647	29,652
SD	1.3	2.5	62.1	132.7	3.8	3.8	12.8	18.9	3216	7217

The delivered dose is the dose that has left the inhaler.
V1 = visit 1 (30 mg inhalation powder); V2 = visit 2 (60 mg inhalation powder).
AUC, area under the concentration time curve; C_{max}, maximum levodopa plasma concentration; SD, standard deviation; T ½ el, elimination half-life time; T_{max}, time to maximum plasma concentration.

After oral administration (100 mg levodopa) the mean C_{max} was 1206.6 ± 448.7 ng/ml. The normalized C_{max} per milligram administered levodopa (calculated from the delivered dose) was 9.10 ng/ml after inhalation of 30 mg, 9.23 ng/ml after inhalation of 60 mg levodopa and 12.06 ng/ml after oral administration.

The AUC per administered mg levodopa is 581.2 ± 127.6 min*ng/ml after inhalation of 30 mg levodopa compared with 574.7 ± 139.9 min*ng/ml after inhalation of 60 mg levodopa. After oral administration, the AUC per mg is 1085.7 ± 296.9 min*ng/ml. The relative bioavailability of inhaled levodopa in comparison with oral levodopa is 53%.

Levodopa plasma concentrations varied strongly after oral administration of levodopa. This also results in large interindividual differences in the T_{max}. The T_{max} after oral administration was 20 min for three participants, 45 min for one participant and 90 min for four participants [mean ± standard deviation (SD): 60 ± 35 min].

A summary of the plasma pharmacokinetic parameters of inhaled levodopa is shown in Table 2. After inhalation of levodopa, there was only a small interindividual variation in the T_{max} for both dose levels, being 5 min for two participants, 10 min for four participants and 15 min for two participants (mean ± SD: 10 ± 4 minutes).

The mean elimination half-life time in our study is 58.3 ± 12.8 min after inhalation of 30 mg, 57.8 ± 18.9 min after inhalation of 60 mg levodopa and 67.7 ± 20.6 min after oral administration of levodopa plus decarboxylase inhibitor.

Inhalation data

Table 3 shows the inhalation data obtained from flow volume curves that were recorded during inhalation of the levodopa by the study participants. The inhaled volumes varied from 1.1 l to 4.2 l (mean ± SD: 2.6 ± 0.75). The maximum pressure drops across the inhaler varied between 1.5 and 10.4 kPa (mean ± SD: 5.8 ± 2.4) with corresponding peak flow rates between 22.9 l/min and 59.9 l/min (mean ± SD: 43.4 ± 9.7). The variation of the inhalation characteristics is larger between the patients than within the patients and can at least partly be explained by differences in sex, age and size of the participants.

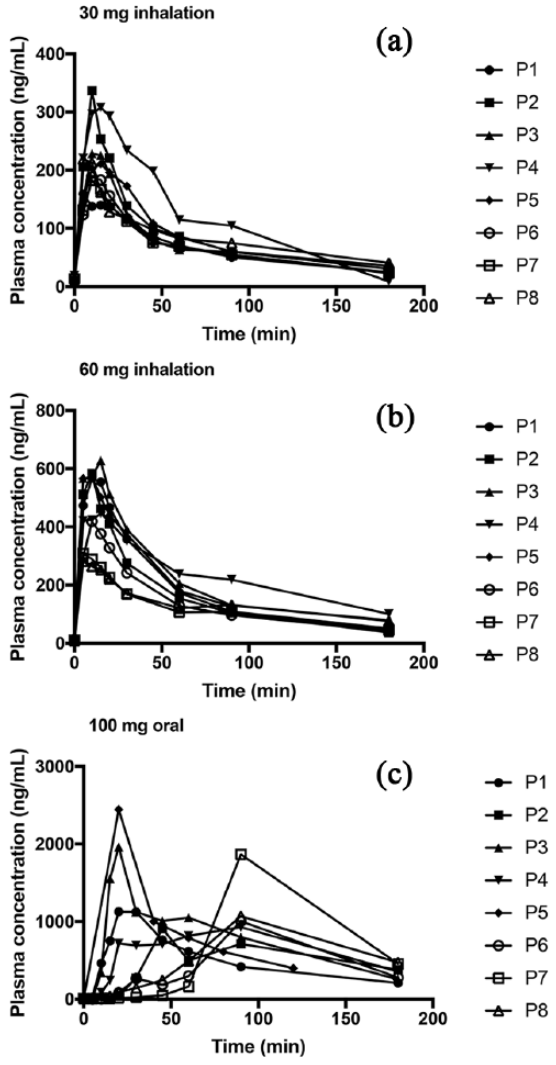

Figure 1. Plasma levodopa concentration (ng/ml) after inhaled or oral levodopa.
Plasma levodopa concentration (ng/ml) after (a) 30 mg inhaled levodopa, (b) 60 mg inhaled levodopa and (c) 100 mg oral levodopa. Individual plasma concentrations of eight patients are shown.

When relating the inhalation data from Table 3 to the plasma pharmacokinetics shown in Table 2, there is no clear relationship between inhaled volumes, attained maximum pressure drops or maximum peak flows and the maximum plasma concentrations that were reached. R^2 values from simple linear regression between these parameters do not exceed 0.022.

Spirometry

No significant change in lung-function parameters (FEV1, FVC, MEF50) was observed after inhalation of either of the levodopa doses or after oral administration of levodopa. None of the patients experienced cough or dyspnoea during or after inhalation.

Discussion

In this study, we assessed the pharmacokinetics and tolerability in Parkinson's disease patients of two doses of levodopa administered *via* a newly developed inhalation system, containing minimal amounts of excipient.

We demonstrated that a levodopa powder formulation with 2% L-leucine administered *via* the Cyclops inhaler is rapidly absorbed into the systemic circulation. In all patients, the T_{max} with levodopa was reached faster after inhalation, that is, within 15 min, whereas after oral administration, T_{max} with levodopa ranged from 20 min to 90 min. The interindividual differences in both the C_{max} and the time to C_{max} were much larger for orally administered levodopa than for inhaled levodopa. In four out of eight patients, it took 90 min to reach C_{max} after oral administration. The slow rise of levodopa plasma concentrations in these patients may be the result of delayed gastric emptying caused by not being in a true fasting state or by Parkinson's disease. It is possible that such a slow rise of the levodopa plasma concentration after oral administration makes oral levodopa less effective for use in an acute setting such as the termination of off periods. In contrast, the results suggest that inhaled levodopa may be much more suitable to terminate an off period because of a consequential rapid rise in the plasma levodopa concentration.

There is no clear relationship between the inhaled volumes, maximal pressure drops, or peak flow rates and the maximal levodopa plasma concentrations that were achieved. This is mostly a consequence of the low variation in delivered dose between the study participants (Table 2). It implies that the combination of inhaler and levodopa formulation results in a robust pulmonary administration not sensitive to differences in inhalation technique or patient characteristics. One should bear in mind, however, that the differences in inhaler technique encountered in this study may not reflect the differences encountered in practice, because of the extensive inhalation instruction given to the study participants.

Table 3. Summary of inhalation parameters obtained from the flow volume curves.

Participant	Inhaled volume (l)			Maximum Δ pressure (kPa)			Peak flow rate (l/min)		
	V1	V2 I1	V2I2	V1	V2I1	V2I2	V1	V2I1	V2I2
1	1.9	2.1	2.1	6.0	6.7	6.7	45.4	47.8	47.9
2	2.7	3.0	3.0	6.8	6.5	6.0	48.4	47.2	45.3
3	2.5	2.1	1.9	4.2	3.9	4.3	37.8	36.6	38.5
4	2.8	2.1	2.7	6.4	6.9	10.0	47.0	48.6	58.7
5	2.5	2.6	2.7	2.6	4.3	4.3	30.0	38.5	38.3
6	4.2	4.2	4.1	10.3	10.4	9.6	59.4	59.9	57.4
7	1.8	1.0	1.8	1.5	2.4	3.7	22.9	28.4	33.7
8	2.8	2.5	2.9	3.7	5.0	6.5	35.8	41.4	47.4

V1 = visit 1; V2I1 = visit 2, inhalation 1; V2I2 = visit 2, inhalation 2.

The relative bioavailability of inhaled levodopa in comparison with oral levodopa is 53%, which is close to the fine particle fraction of the delivered dose of 45% and therefore appears to reflect the lung deposition fraction. After all, for effective deposition of inhalation powder in the airways, and thus absorption in the systemic circulation, a particle size between 1 and 5 µm is required.[25] However, the bioavailability of inhaled levodopa in this study is likely lowered by the absence of a decarboxylase inhibitor in the formulation. The levodopa inhalation powder does not contain a decarboxylase inhibitor, since its intended future use is on an 'as needed' basis as rescue medication on top of oral levodopa administered together with a decarboxylase inhibitor as maintenance therapy. Since the participants in this study had to postpone their own levodopa with decarboxylase inhibitor at least five half-life times before administration of the study levodopa, the pharmacokinetics of oral levodopa plus decarboxylase inhibitor is compared with that of inhaled levodopa without decarboxylase inhibitor. Therefore, the AUC of inhaled levodopa will be higher when used on an 'as needed' basis on top of maintenance therapy due to decreased peripheral conversion of levodopa to dopamine.[14] Because the relative bioavailability is higher than the fine particle fraction (i.e. the fraction suitable for deep lung deposition), our results imply that for effective absorption into the systemic circulation, deposition of levodopa particles does not necessarily need to be in the peripheral airways. This adds to the robustness of this route of administration.

The calculated elimination half-life for inhaled levodopa varied between 34 min and 93 min. The mean elimination half-life we found in this study was 58 min after inhalation of levodopa and 68 min after oral administration of levodopa plus decarboxylase inhibitor. This is shorter than the half-life of 90 min previously described in literature.[15] Several previous studies have already confirmed that the pharmacokinetics of levodopa display considerable interparticipant variation,[14] which in our study, is the case for AUC, C_{max} and elimination half-life time. Therefore, for a more accurate assessment of these parameters, a study population larger than eight patients would be desirable.

In none of the patients, a drop in FEV1, FVC or MEF50 was observed. Furthermore, none of the patients experienced cough or dyspnoea during or after the inhalation manoeuvre. In the study reported by Lipp and colleagues,[18] 21.7% of the patients reported cough after inhalation of their levodopa formulation. A common cause for cough after inhalation is the deposition of drug particles in the oropharynx. We assume that due to the high airflow resistance of the Cyclops inhaler,[21] deposition of levodopa in the oropharynx is prevented, which explains the absence of

cough after the levodopa inhalation formulation used in this study. Another reason for cough after inhalation is the chemical composition of the powder.[26] Our inhalation powder consists of only 2% excipient. Since coughing is possibly a reason for patients to withdraw inhalation therapy, the absence of cough is an important advantage of the formulation used in this study.

Whether or not the levodopa plasma concentrations attained by inhalation in this study are sufficient for rescue therapy in off periods will depend on disease progression and the degree to which a patient is turned 'off'. In progressed, fluctuating patients, a very steep dose–response relationship exists, where a maximum effect on finger tapping can be achieved by an increase in levodopa effect compartment concentration of approximately 200–400 ng/ml.[27] Therefore, the plasma concentrations attained by inhalation of levodopa in this study (i.e. 229 ng/ml with 30 mg and 476 ng/ml with 60 mg) could be clinically sufficient to end off episodes in Parkinson's disease. Because the study participants were not in the off state before levodopa inhalation, no effect could be observed. In a follow-up clinical trial, we will study the effect of inhaled levodopa from the Cyclops DPI on Parkinson's disease patients in the 'off state' in comparison with orally administered levodopa. This will show whether or not the faster absorption after inhalation is of clinical benefit.

Conclusion

Oral administration results in a more variable levodopa plasma profile than pulmonary administration. In addition, inhaled levodopa is absorbed up to 85 min faster in the blood plasma and inhaled doses of 30 mg and 60 mg showed comparable pharmacokinetics per milligram of inhaled levodopa. Since none of the patients experienced cough or dyspnoea and no change in pulmonary function was measured, it is concluded that the new levodopa powder inhalation system is well tolerated after inhalation. The results of this study therefore suggest that the tested levodopa formulation may be particularly beneficial for use during an off period, since a rapid onset of action without any harmful effects are two key requirements for such a rescue medication. Furthermore, no relationship was found between inhalation parameters, such as inhaled volume and inhalation flow rate, and levodopa pharmacokinetic parameters, which is indicative of a robust administration method. A study evaluating the efficacy of inhaled levodopa from the Cyclops for Parkinson's patients in an off period will be performed next.

Acknowledgements

The authors thank Ms L Koopmans and Ms F Wester-Vast for performing the spirometry measurements and Ms W Bossen, E Hoeksema, D Seigers and Mr W Wiersema for drawing the blood samples.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was funded by a research grant from the Dutch Parkinson Society 'Parkinson Vereniging'.

Conflict of interest statement

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: The employer of PH, AB and HWF receives royalties from the sales of the Cyclops DPI. FG is currently partly employed by PureIMS BV, the manufacturer of the Cyclops DPI.

ORCID iD

Floris Grasmeijer https://orcid.org/0000-0003-0897-5673

References

1. Bergman H and Deuschl G. Pathophysiology of Parkinson's disease: from clinical neurology to basic neuroscience and back. *Mov Disord* 2002; 17(Suppl. 3): S28–S40.
2. Postuma RB. MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord* 2015; 30: 1591–1601.
3. Fox SH. International Parkinson and movement disorder society evidence-based medicine review: update on treatments for the motor symptoms of Parkinson's disease. *Mov Disord* 2018; 33: 1248–1266.
4. Fernandez H, Vanagunas A, Odin P, *et al.* Levodopa-carbidopa intestinal gel in advanced Parkinson's disease open-label study: interim results. *Parkinsonism Relat Disord* 2013; 19: 339–345.

5. Doi H, Sakakibara R, Sato M, *et al.* Plasma levodopa peak delay and impaired gastric emptying in Parkinson's disease. *J Neurol Sci* 2012; 319: 86–88.
6. Cenci MA, Ohlin KE and Odin P. Current options and future possibilities for the treatment of dyskinesia and motor fluctuations in Parkinson's disease. *CNS Neurol Disord Drug Targets* 2011; 10: 670–684.
7. Pedrosa DJ and Timmermann L. Review: management of Parkinson's disease. *Neuropsychiatr Dis Treat* 2013; 9: 321–340.
8. Chen JJ and Obering C. A review of intermittent subcutaneous apomorphine injections for the rescue management of motor fluctuations associated with advanced Parkinson's disease. *Clin Ther* 2005; 27: 1710–1724.
9. Haq IU, Lewitt PA and Fernandez H. Apomorphine therapy in Parkinson's disease: a review. *Expert Opin Pharmacother* 2007; 8: 2799–2809.
10. Jankovic J and Stacy M. Medical management of levodopa-associated motor complications in patients with Parkinson's disease. *CNS Drugs* 2007; 21: 677–692.
11. Robertson DR, Wood ND and Everest H. The effect of age on the pharmacokinetics of levodopa administered alone and in the presence of carbidopa. *Br J Clin Pharmacol* 1989; 28: 61–69.
12. Nutt J JG. The effect of carbidopa on the pharmacokinetics of intravenously administered levodopa: the mechanism of action in the treatment of parkinsonism. *Ann Neurol* 1985; 11: 537–43.
13. Cedarbaum JM. Effect of supplemental carbidopa on bioavailability of L-dopa. *Clin Neuropharmacol* 1986; 9: 153–159.
14. LeWitt PA. Levodopa therapy for Parkinson's disease: pharmacokinetics and pharmacodynamics. *Mov Disord* 2015; 30: 64–72.
15. Nyholm D. Pharmacokinetic optimisation in the treatment of Parkinson's disease: an update. *Clin Pharmacokinet* 2006; 45: 109–136.
16. Patton JS. The lungs as a portal of entry for systemic drug delivery. *Proc Am Thorac Soc* 2004; 1: 338–344.
17. Patton JS. Pulmonary polypeptide and polynucleic acid delivery mechanisms of macromolecule absorption by the lungs. *Adv Drug Deliv Rev* 1996; 19: 3–36.
18. Lipp M. Preclinical and clinical assessment of inhaled levodopa for off episodes in Parkinson's disease. *Sci Transl Med* 2016; 8: 360ra136.
19. Luinstra M. Can patients with Parkinson's disease use dry powder inhalers during off periods? *PLoS One* 2015; 10: e0132714.
20. Luinstra M. A levodopa dry powder inhaler for the treatment of Parkinson's disease patients in off periods. *Eur J Pharm Biopharm* 2015; 97: 22–29.
21. Hoppentocht M, Akkerman OW, Hagedoorn P, *et al.* The Cyclops for pulmonary delivery of aminoglycosides; a new member of the Twincer™ family. *Eur J Pharm Biopharm* 2015; 90: 8–15.
22. Miller MR, Hankinson J, Brusasco V, *et al.* Standardisation of spirometry. *Eur Respir J* 2005; 26: 319–338.
23. Quanjer PH. Lung volumes and forced ventilatory flows. Report working party standardization of lung function tests, european community for steel and coal. Official statement of the European Respiratory Society. *Eur Respir J* 1993; 16: 5–40.
24. Borg GA. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 1982; 14: 377–381.
25. McElroy MC. Inhaled biopharmaceutical drug development: nonclinical considerations and case studies. *Inhal Toxicol* 2013; 25: 219–232.
26. Hoppentocht M. Tolerability and pharmacokinetic evaluation of inhaled dry powder tobramycin free base in non-cystic fibrosis bronchiectasis patients. *PLoS One* 2016; 11: e0149768.
27. Contin M. Pharmacodynamic modeling of oral levodopa: clinical application in Parkinson's disease. *Neurology* 1993; 43: 367–371.